

Catalase and Superoxide Dismutase Activities after Heat Injury of *Listeria monocytogenes*

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Four strains of *Listeria monocytogenes* were examined for catalase (CA) and superoxide dismutase (SOD) activities. The two strains having the highest CA activities (LCDC and Scott A) also possessed the highest SOD activities. The CA activity of heated cell extracts of all four strains examined decreased sharply between 55 and 60°C. SOD was more heat labile than CA. Two *L. monocytogenes* strains demonstrated a decline in SOD activity after heat treatment at 45°C, whereas the other two strains demonstrated a decline at 50°C. Sublethal heating of the cells at 55°C resulted in increased sensitivity to 5.5% NaCl. Exogenous hydrogen peroxide was added to suspensions of *L. monocytogenes*; strains producing the highest CA levels showed the greatest H₂O₂ resistance.

Listeria monocytogenes is a facultative intracellular pathogen recently implicated in several fatal foodborne disease outbreaks. The organism has been reported to survive milk pasteurization under certain conditions (3). The univalent reduction of molecular oxygen (O₂) results in the production of the toxic intermediate species: the superoxide radical (O₂⁻), hydrogen peroxide (H₂O₂), and the hydroxyl radical (OH·). Two listerial enzymes involved in the detoxification of O₂⁻ and H₂O₂ are superoxide dismutase (SOD) and catalase (CA).

L. monocytogenes ATCC 7644 and ATCC 15313 were obtained from the American Type Culture Collection, Rockville, Md.; strains LCDC and Scott A were obtained from Larry Beuchat, University of Georgia Experimental Station, Experiment, Ga. Cells were grown overnight in tryptic soy broth at 37°C with shaking, concentrated by centrifugation (10,000 × g, 10 min), and washed in 50 mM potassium phosphate buffer, pH 7.0. Cell extracts were prepared by passing cells through a French pressure cell (American Institute Corp., Silver Spring, Md.) at a pressure of 7,600 lb/in². The resulting lysate was centrifuged at 16,300 × g for 10 min at 4°C, and the supernatant was retained. CA activity was determined by the spectrophotometric method of Beers and Sizer (2). One unit of CA decomposes 1.0 μmol of H₂O₂ per min at 25°C at pH 7.0, whereas the H₂O₂ concentration falls from 10.3 to 9.2 μmol/ml of reaction mix. SOD activity was measured by the cytochrome *c* reduction method of McCord and Fridovich (9). Protein concentration was determined by the method of Lowry et al. (6), with lysozyme as the standard. Cell extracts were heated in test tubes (13 by 100 mm) for 10 min in water baths and cooled in crushed ice. For heat injury, *L. monocytogenes* was grown in tryptic soy broth at 35°C for 12 h, pelleted by centrifugation, and suspended in an equal volume of 100 mM phosphate buffer, pH 7.2. A 10-ml portion of this suspension was added to 90 ml of 100 mM phosphate buffer, tempered to 55°C, and heated with constant stirring for specified periods of time. After being heated, the suspension was immediately cooled and plated on tryptic soy agar (TSA) to enumerate all viable cells and on TSA plus 5.0% NaCl (TSAS) to enumerate uninjured cells. The difference between these two counts

represents the number of injured cells. Unheated cells were also plated on these media. Plates were incubated for 48 h at 35°C. Strains of *L. monocytogenes* were exposed to exogenous H₂O₂ by placing 1 ml of a dilution of an overnight culture (ca. 10⁶ cells) in 9.0 ml of 0.1% peptone-water containing H₂O₂ at a final concentration of 100 mM. A second portion was transferred to 0.1% sterile peptone-water containing 0.02% CA (3,400 U/ml). The mixtures were held at 25°C for appropriate time intervals and then added to 0.1% peptone water containing 0.02% CA. These cells were then spread-plated on TSA, and the plates were incubated for 48 h at 35°C.

The specific activities of CA and SOD in extracts of the four strains of *L. monocytogenes* examined are presented in Table 1. Two of the listerial strains (LCDC and Scott A) had CA levels comparable to those of other pathogens. For example, *Escherichia coli* ATCC 29682 and *Mycobacterium intracellulare* have CA specific activities of 28.2 and 34.7 U/mg of protein, respectively (4, 7). The SOD levels produced by strains Scott A and LCDC were higher than those produced by other pathogens. *E. coli* ATCC 29682 and *M. intracellulare* had SOD activities of 25.9 and 22.9 U/mg of protein, respectively (4, 8). Two of the listerial strains examined (ATCC 7644 and ATCC 15313) had lower levels of both CA and SOD relative to those of strains LCDC and Scott A.

The specific activities of CA and SOD in heated cell extracts of *L. monocytogenes* are presented in Fig. 1 and 2. All four strains retained 81 to 86% of their initial CA levels through 55°C (Fig. 1). At 60°C, all four strains demonstrated

TABLE 1. CA and SOD specific activities^a and T₅₀s of *L. monocytogenes* cell extracts

Strain	CA sp act ^b	CA T ₅₀	SOD sp act ^c	SOD T ₅₀
ATCC 7644	5.5	57.5	33.0	53.5
ATCC 15313	10.6	58.0	28.0	56.0
LCDC	20.7	59.0	110.0	52.5
Scott A	24.5	59.0	134.0	52.0

^a Specific activities are reported as units of CA or SOD per milligram of protein.

^b One unit of CA decomposes 1.0 μmol of H₂O₂ per min.

^c One unit of SOD is defined as the amount of SOD required to inhibit the rate of reduction of cytochrome *c* by 50%.

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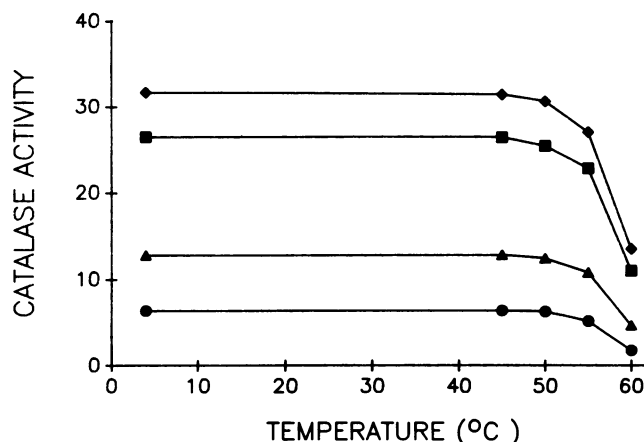


FIG. 1. CA specific activity (units per milligram of protein) measured after heating the *L. monocytogenes* cell extracts for 10 min. Symbols: ●, ATCC 7644; ▲, ATCC 15313; ■, LCDC; ◆, Scott A.

a sharp drop in CA levels. The strain which produced the least CA, ATCC 7644, retained 27% of its original activity, whereas strain Scott A, which produced the most CA, retained 42% of its initial CA activity after 10 min at 60°C. The temperature at which 50% of the enzyme activity was lost (T_{50}) for all four strains (CA) ranged between 57.5 and 59.0°C (Table 1). These CA T_{50} s are lower than those reported for *Staphylococcus aureus* (1). SOD was more sensitive to heat. Two strains (ATCC 7644 and ATCC 15313) retained SOD activity through 55°C (Fig. 2). Strains LCDC and Scott A, having initially high SOD activities, quickly lost SOD activity when heated. The SOD T_{50} for all strains ranged from 52.0 to 56.0°C (Table 1).

Heat resistance of whole listerial cells was examined (Table 2). All four strains demonstrated increased sensitivity to NaCl, as shown by the difference in counts on TSA and TSAS after the cells were heated. There was no apparent correlation between CA or SOD levels and resistance to heat

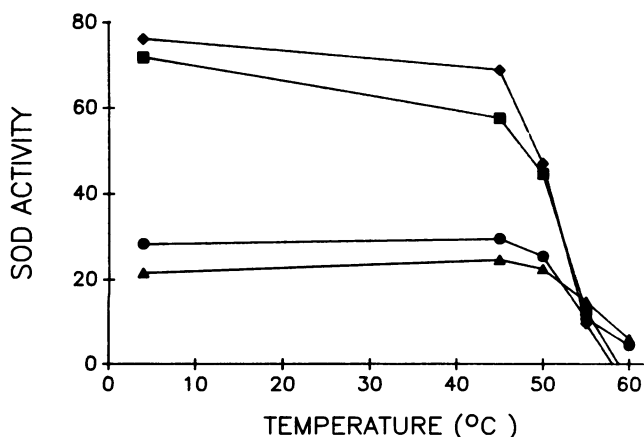


FIG. 2. SOD specific activity (units per milligram of protein) measured after heating the *L. monocytogenes* cell extracts for 10 min. Symbols: ●, ATCC 7644; ▲, ATCC 15313; ■, LCDC; ◆, Scott A.

TABLE 2. Comparative heat injury^a of *L. monocytogenes*

Strain	Min at 55°C	Log CFU/ml (% enumeration ^b) on:			
		TSA	TSAS	TSA-HI ^c	TSAS-HI ^c
ATCC 7644	35	9.5 (100)	9.4 (96)	8.9 (26)	5.8 (0.01)
ATCC 15313	10	9.3 (100)	9.1 (64)	8.8 (28)	5.6 (0.02)
LCDC	30	9.4 (100)	9.3 (92)	8.8 (24)	6.0 (0.04)
Scott A	20	9.3 (100)	9.3 (90)	8.4 (11)	4.7 (0.002)

^a Cells were heated at 55°C in 100 mM phosphate buffer.

^b Percent enumeration on TSA was defined as 100.

^c HI, Heat-injured cells.

TABLE 3. Effects of H₂O₂ on the survival of *L. monocytogenes*^a

Exposure time (min)	% Survival ^b of strain:			
	7644	15313	LCDC	Scott A
5	68	57	83	81
10	47	31	68	70
15	16	9	32	62

^a Cells were exposed to exogenous 100 mM H₂O₂ and enumerated on TSA.

^b At time zero, survival was defined as 100% for each strain.

injury. Neither the addition of pyruvate or exogenous CA nor the anaerobic incubation of cells increased the number of uninjured cells on either TSA or TSAS (data not shown).

As expected, exposure of *L. monocytogenes* cells to exogenous H₂O₂ resulted in lower survival values for the low-CA-producing strains than for the high-CA-producing strains (Table 3). This observation is consistent with those survival values found for *E. coli* (5).

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